

Basophil Activation Test: Methods

Basophil Activation Test (BAT) was performed according to a previously reported technique [E1]. Briefly, endotoxin-free heparinized whole-blood samples were obtained from the allergic patients and a healthy control. Cells were challenged with 100 µl of anti-IgE (10 µg/ml; clone G7-18; BD Bioscience, USA), allergens (donkey raw milk diluted 1:50 in buffer; cow milk extract from prick test, Lofarma, Italy, undiluted), fMLP (0.5 µg/ml; Sigma Aldrich, Italy) for 20 min at 37 ° C in a water bath (optimal stimulation times were assessed in previous experiments). As a negative control, Tyrode solution (Sigma Aldrich) with 20 µ M HEPES and 7.5% NaHCO₃, pH 7.4, was used to assess the spontaneous expression of the different markers. The reactions were terminated by chilling the cells on ice.

Immunophenotyping and Flow-Cytometric Analyses

Basophils were stained with 5 µl of anti-human CD3 Pacific Blue (Beckman Coulter, USA), 10 µl of anti-human CD63-FITC (clone H5C6; BD Biosciences), 10 µl of anti-human CD203c-APC (clone NP4D6; BioLegend) and 10 µl of monoclonal anti-human CD294 (CRTH2) PE (Beckman Coulter, USA) for 15 min on room temperature. Flow-cytometric analyses were performed on a NAVIOS flow cytometer (Beckman Coulter, USA). We gated on physical parameters forward (FS) and side (SS) scatter to exclude debris. We then gated on CRTH2 (CD294) positive/CD3 negative cells to isolate basophils.

Reference:

1. Hoffmann HJ, Santos AF, Mayorga C, Nopp A, Eberlein B, Ferrer M et al. The clinical utility of basophil activation testing in diagnosis and monitoring of allergic disease. *Allergy*. 2015 Nov;70(11):1393-405.